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<p>(54) Title: NANOMETER SIZED PARTICLES CONTAINING A REACTIVE MONOLAYER ADSORBED THEREON AND METHODS OF MAKING THE SAME</p> <p>(57) Abstract</p> <p>A nanometer-sized particle comprises a core comprising at least one metal or metal alloy; and a monolayer chemically bonded to the core. The monolayer contains at least one reactive substituent which is coupled to a functional material such that the monolayer is chemically modified. A method of making a functionalized nanometer-sized particle comprises providing a nanometer-sized particle comprising a core which comprises at least one metal or metal alloy, and a monolayer adsorbed onto the core wherein the monolayer includes at least one reactive substituent; and coupling the nanometer-sized particle with a functional material such that the monolayer is chemically modified.</p>			

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15 **NANOMETER SIZED PARTICLES CONTAINING A REACTIVE MONOLAYER  
ADSORBED THEREON AND METHODS OF MAKING THE SAME**Field of the Invention

The invention generally relates to nanometer-sized particles which have been chemically modified and methods of making the same.

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Background of the Invention

Technology involving the synthesis of nanometer-sized particles ("nanotechnology") has gained widespread attention recently. Broadly speaking, nanotechnology relates to the art and science of building molecular materials so that they are capable of functioning as macro-scale structures and/or exhibiting physical and chemical properties which are intermediate between molecular and bulk materials. Applications involving nanotechnology are potentially far reaching. Areas of possible interest relate to, for example, catalysis, molecular electronics, biotechnology, composite materials, solar energy conversion, and the like. Investigative efforts regarding nanotechnology have focused largely on understanding the physical behavior and structure of nanometer-sized materials. Reiss, H., *Proceedings of the Welch Foundation 39<sup>th</sup> Conference on Chemical Research: Nanophase Chemistry* (1995) 49-66 discusses thermodynamic behavior associated with nanophase technology. Berry, R.S., *Proceedings of the Welch Foundation 39<sup>th</sup> Conference on Chemical Research: Nanophase*

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Chemistry, (1995), 71-81 focuses on the surface behavior of nanometer-sized particles. Quate, C.F., *Proceedings of the Welch Foundation 40<sup>th</sup> Conference on Chemical Research: Nanophase Chemistry* (1996) 87-95, discusses the use of a scanning probe lithography in fabricating nanostructures. Schön, G., et al., *Colloid Polym. Sci.* **273** (1995) 202-218, discusses uses of nanometer-sized particles relevant to the microelectronics industry.

Nanometer-sized gold particles can be chemically attached to metal surfaces, such as electrodes. The purpose of such experiments is to allow the researcher to add functionality onto the immobilized particles in order to add value to the metal surface. For example, Freeman, R.G., et al., *Science* **267** (1995) 1629-1632, propose linking gold and silver colloidal particles to a silanized surface. Doron, A., et al., *Langmuir* **11** (1995) 1313-1317, proposes linking gold colloid particles to an indium tin oxide surface, followed by further functionalizing the exposed edge of the bound colloid particles with electroactive functional groups. The aforementioned techniques may be limited to surface immobilized particles which makes characterization of the end-functionalized material difficult and uncertain. Furthermore, there is a potential general constraint in that the size of the particle attached to the surface is significantly less able to participate in the functionalization reactions. In essence, the aforementioned work may be useful only as a means to add value to a large (millimeter or micrometer scale) surface.

Metal sols are small particles which are insoluble, and thus suspended, in the liquid in which they are dispersed. Numerous recent studies have investigated methods for adding to the complexity of metal sols. For example, Mirkin, C.A., et al., *Nature* **382** (1996) 607-609, proposes attaching oligonucleotides to gold sols in order to promote aggregation of said sols upon addition of an appropriate complementary oligonucleotide to the sol solution. U.S. Patent No. 4,859,612, to Cole et al. proposes that antibody coated metal sols can interact with an appropriately coated solid phase particle as a means for an immunoassay procedure. U.S. Patent Nos. 5,294,369 and 5,384,073 to

Shigekawa et al. propose that gold sols can be mixed in an alcohol solution containing alkanethiol derivatives. Upon isolation of the particles, antigens, antibodies, and ligands can be further linked to the gold particle via a reaction in an aqueous buffer. In all of the aforementioned examples, the proposed 70 methodology may be limited to metal sols, reactions in heterogeneous media, and a specific subset of functionalized materials. The constraints of the aforementioned methods potentially make analysis of the monolayer difficult and unreliable.

In addition to the above, recent efforts have focused on producing 75 nanometer-sized particles having other materials chemically or physically attached thereto. For example, Brust, M., et al., *J. Chem. Soc, Chem. Commun.* (1994) 801-802, proposes nanometer-sized gold cores which are stabilized by chemisorbed layers of dodecanethiolate. The resulting material is soluble in non-polar organic solvents and can be repeatedly isolated and reused. Terrill, R. 80 H., et al., *J. Am. Chem. Soc.*, **117:50** (1995) 12537-12548, proposes nanometer-sized gold cores which are stabilized by chemisorbed layers of octane- or hexadecanethiolate. These monolayer-protected gold clusters were found to be highly stable as determined by differential scanning calorimetry techniques. Hostetler, M.J., et al., *Langmuir*, **12** (1996) 3604-3612, relates to the evaluation 85 of the physical structure of alkanethiolates of various chain lengths adsorbed onto nanometer-sized gold cores. Alkanethiolates with shorter chain lengths were determined to be relatively disordered while materials with longer chain lengths were found to be in the trans zig-zag conformation. The previous three descriptions of the art represent nanometer-sized gold cores covered with 90 simple, non-derivatized alkanethiols, a circumstance that severely limits their applicability.

Hostetler, M.J., et. al., *J. Am. Chem. Soc.*, **118** (1996) 4212-4213 proposes nanometer-sized gold cores stabilized by mixed monolayers of unsubstituted and  $\omega$ -substituted (cyano, bromo, vinyl, ferrocenyl) alkanethiolates. 95 A potential limitation of this and other methods are that they typically rely on

either commercially available or easily-prepared  $\omega$ -substituted alkanethiols. Another deficiency in these methods is that formation of the  $\omega$ -substituent often does not occur on the protected gold cores, causing the need for extensive purification steps that can only produce a narrow range of compounds.

100 Notwithstanding the above, there is a need in the art for nanometer-sized materials which exhibit specific chemical and physical properties. More particularly, there is a need for such materials which can be tailored for utilization in a number of defined end use applications. It would be particularly desirable if the nanometer-sized particles exhibited flexible chemical behavior as well as 105 multiplicity with respect to reactivity. It would also be particularly worthwhile if the reverse engineering needed to design the synthesis of an envisioned multi-functionalized nanometer-sized particle would be straightforward and understandable. It would be notably beneficial if the reactions forming the particles were homogeneous, flexible, easy to learn, and reliable. This would be especially advantageous in analytical applications involving the solubilization of 110 the nanometer-sized particles in aqueous or organic solvents.

#### Summary of the Invention

115 It is therefore an object of the present invention to provide nanometer-sized particles which may be chemically tailored to be desirable in a variety of applications.

120 These and other objects and features are provided by the present invention. In one aspect, the invention relates to a functionalized nanometer-sized particle comprising a core which comprises at least one metal or metal alloy; and a monolayer chemically bonded to the core. The monolayer is formed during the formation of the core and can be modified at any time following formation of the core. Advantageously, the monolayer contains at least one reactive substituent thereon which is coupled to a functional material such that the monolayer becomes chemically modified. The reactive substituent may be 125 selected from a number of groups such as, for example, SH, OH, NH<sub>2</sub>, NH,

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CO<sub>2</sub>H, SO<sub>3</sub>OH, PO<sub>2</sub>(OH)<sub>2</sub>, BO(OH)<sub>2</sub>, or mixtures thereof. By virtue of the nanometer-sized particle structure, a number of functional materials may be employed such as catalysts, biomaterials, and materials which are chemically, electrochemically, or photochemically active. Thus, the particles may be readily used in a number of applications.

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In another aspect, the invention relates to a method of making a functionalized nanometer-sized particle. The method comprises providing a nanometer-sized particle comprising: (1) a core which comprises at least one metal or metal alloy and (2) a monolayer chemically bonded to the core, the monolayer including at least one reactive substituent. The nanometer-sized particle is then coupled with a functional material such that the monolayer becomes chemically modified. Importantly, this method allows for the particle to be modified based on a small subset of reactive substituents, all of which can be readily synthesized or purchased commercially. Adding greater value to said nanometer-sized particle can then be accomplished using a variety of functional materials.

#### Brief Description of the Drawings

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**FIG. 1** is a representation of a multi-step synthesis of a tripeptide-functionalized monolayer-protected nanometer-sized gold core.

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**FIG. 2a** is a graph illustrating the electrochemical characterization of a 10H-(phenothiazine-10)propionic acid-functionalized monolayer-protected nanometer-sized gold core. Specifically, the figure illustrates the cyclic voltammetry of 0.8 mM 10H-(phenothiazine-10)propionic acid (--) in 2:1 toluene/acetonitrile (v/v) at 100mV/s; and

**FIG. 2b** is a graph illustrating the thin layer coulometry charge Q vs. cell length (L) ( $r^2=0.98$ ; slope=11.47 X 10<sup>-3</sup> C/cm).

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Detailed Description of the Preferred Embodiments

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The present invention now will be described more fully hereinafter with reference to the accompanying specification, examples, and drawings, in which preferred embodiments of the invention are shown. This invention may, however, be embodied in many different forms and should not be construed as limited to the embodiments set forth herein. Rather, these embodiments are provided so that this disclosure will be thorough and complete, and will fully convey the scope of the invention to those skilled in the art.

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In one aspect, the invention relates to a functionalized nanometer-sized particle. The functionalized nanometer-sized particle comprises a core comprising at least one metal or metal alloy. For the purposes of the invention, the core preferably has a diameter ranging from about 1 nm to about 999 nm, more preferably from about 1 nm to about 100 nm; even more preferably from about 1 nm to about 20 nm; and most preferably from about 1 nm to about 7 nm. A monolayer is chemisorbed or chemically bonded to the core. The monolayer contains at least one reactive substituent as described further herein. Advantageously, the reactive substituent(s) on the monolayer is/are coupled to a functional material so as to chemically modify the monolayer.

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A number of metals and metal alloys may be used in the core. Preferably, the metal or metal alloy is selected from the group consisting of a semiconducting material, a metal oxide material, a Group VIIIA element, a Group IB element, a Group IIB element, alloys thereof, and mixtures thereof. More preferably, the metal or metal alloy is selected from the group consisting of a Group VIIIA element, a Group IB element, alloys thereof, and mixtures thereof. Examples of elements which may be used include, but are not limited to, gold, silver, copper, palladium, platinum, nickel, and alloys thereof. Examples of semiconducting materials include, but are not limited to, cadmium sulfide, indium phosphide, and other Group III-V materials. Examples of oxide materials include, but are not limited to, titanium oxide (titania), aluminum oxide (alumina), tin oxide, and iron oxide. The shape of the core is not restricted to any particular

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185 geometry, thus, for example, rods, spheres, cuboctahedra, and truncated octahedra, will all satisfy the conditions stated herein.

For the purposes of the invention, the term "monolayer" may be defined as a layer preferably having a thickness ranging from about 0.4 nm to about 100 nm, and more preferably 1.0 to 20 nm. Although not wishing to be bound by any one embodiment, the monolayer is typically formed during the formation of the core, and the monolayer can be modified at any point following formation of the core. The monolayer which is adsorbed or chemically bonded to the core may comprise a number of materials. Examples of these materials include, but are not limited to, organic compounds (e.g., alkanethiols, arylthiols, vinylthiols, and their derivatives); inorganic compounds (e.g., alkyl borates, alkyl phosphonates, alkyl silicates), organometallic compounds (e.g., ferrocenethiol); biochemical compounds (e.g., cysteine, albumin, coenzyme A), and mixtures thereof.

200 In referring to the molecular structure of the monolayer, substituents which may be present on the monolayer include, for example, branched molecules (e.g., branched alkyl chains, multiply substituted aryl groups, multiply substituted cyclic aliphatic compounds), hyperbranched molecules (e.g., dendritic molecules); and mixtures thereof. As an example of a non-branched substituent, the monolayer may comprise an alkanethiol or an alkanethiol derivative.

205 Exemplary alkanethiols include those having between 2 and 23 carbon atoms.

210 As alluded to herein, the monolayer contains at least one reactive substituent. For the purposes of the invention, the term "reactive substituent" refers to those substituents which are chemically active so that, upon reaction with a functional material, part of the reactive substituent remains with the product, serving as a linking or coupling group between the original part of the molecule and the new portion of the molecule. Examples of reactive substituents include, but are not limited to, SH, OH, NH<sub>2</sub>, NH, CO<sub>2</sub>H, SO<sub>3</sub>OH, PO<sub>2</sub>(OH)<sub>2</sub>, BO(OH)<sub>2</sub>, and mixtures thereof. More preferably, OH, NH, CO<sub>2</sub>H, NH<sub>2</sub>, and

215 mixtures thereof are employed. Examples of compounds which may be present on the reactive substituent are described by the general formula:



220 wherein R is selected from the group consisting of an organic compound, an inorganic compound, an organometallic compound, a biochemical compound, and mixtures thereof; E is selected from the group consisting of S, O, NH, COO, SO<sub>3</sub>OH, PO<sub>2</sub>(OH)<sub>2</sub>, BO(OH)<sub>2</sub>, CO<sub>2</sub>, NH<sub>2</sub>, and mixtures thereof; n is an integer ranging from 1 to 5 (more preferably from 1 to 2); and x is an integer ranging from 1 to 10 (more preferably from 1 to 3). More preferably, E is selected from O, NH, CO<sub>2</sub>, NH<sub>2</sub>, and mixtures thereof. The chemically modified monolayer may also include partially reactive and nonreactive compounds or materials.

225 Compounds or materials being "partially reactive" refer to those containing groups which do not participate in coupling but instead are capable of undergoing substitution reactions, elimination reactions, oxidative and reductive reactions, and the like. "Nonreactive" compounds or materials refer to those which do not undergo the above reactions. Reactive, partially reactive, and

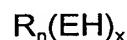
230 nonreactive compounds or materials may be used in any ratio so long as at least one reactive compound or material is present on the monolayer.

235 The monolayer is preferably chemically bonded to the core by various types of bonds. Examples of these bonds include, but are not limited to, core-element-sulfur bonds, core-element-oxygen-bonds, core-element-boron, core-element-phosphorus, and core-element-nitrogen-bonds. Combinations of these types of bonds may also be used. In the most preferred embodiment the monolayer is chemically bonded to the core by various core-element-sulfur bonds. In the aforementioned embodiments, core-element refers to any element of which the core is composed.

240 In accordance with the invention, the monolayer is coupled to a functional material such that the monolayer is chemically modified. The term "coupled" may be interpreted to mean that the monolayer and the functional material are linked via formation of a new chemical bond. Examples, include, but are not

limited to amide, thioester, and ester-forming reactions known in the art.

245 Examples of functionalities which may be present on the functional materials include, but are not limited to, SH, OH, NH<sub>2</sub>, NH, CO<sub>2</sub>H, SO<sub>3</sub>OH, PO<sub>2</sub>(OH)<sub>2</sub>, BO(OH)<sub>2</sub>, and mixtures thereof. Examples of reactive substituents include, but are not limited to, SH, OH, NH<sub>2</sub>, NH, CO<sub>2</sub>H, SO<sub>3</sub>OH, PO<sub>2</sub>(OH)<sub>2</sub>, BO(OH)<sub>2</sub>, and mixtures thereof. More preferably, OH, NH, CO<sub>2</sub>H, NH<sub>2</sub>, and mixtures thereof are employed. The functional material may also comprise at least one 250 compound having the general formula:



wherein R is selected from the group consisting of an organic compound, an inorganic compound, an organometallic compound, a biochemical compound, 255 and mixtures thereof; E is selected from the group consisting of S, O, NH, COO, SO<sub>3</sub>OH, PO<sub>2</sub>(OH)<sub>2</sub>, BO(OH)<sub>2</sub>, CO<sub>2</sub>, NH<sub>2</sub>, and mixtures thereof; n is an integer ranging from 1 to 5 (more preferably from 1 to 2); and x is an integer ranging from 1 to 10 (more preferably from 1 to 3). More preferably, E is selected from O, NH, CO<sub>2</sub>, NH<sub>2</sub>, and mixtures thereof. It should be emphasized that the 260 functional material may be present in the form of a number of structures which possesses functionality in the manner described herein. For example, the functional material may be a catalyst, a biomaterial, a material which is electrochemically active, or combinations thereof. The functional material may also be one which has a low-lying excited state which is capable of undergoing 265 fluorescence or electron-transfer when excited. The functional material may also be selected such that the nanometer-sized particle is soluble in a solvent. The term "soluble" may be defined to mean the particles being dispersed or dissolved in the solvent. Examples of suitable solvents include aqueous or organic solvents.

270 In another aspect, the invention relates to a method of making a functionalized nanometer-sized particle. The method comprises providing a nanometer-sized particle comprising a core which comprises at least one metal or metal alloy, and a monolayer adsorbed onto the core. The monolayer

275 includes at least one reactive substituent. The nanometer-sized particle is then coupled with a functional material such that the monolayer is chemically modified.

A number of functional materials may be used in the above method. Examples of these materials include, but are not limited to, spin labels (e.g., 4-amino-TEMPO), metal ligands (e.g., 4-(aminomethyl)-pyridine), amino acids (e.g., glutamic acid *di*-*t*-butyl ester), chromophores and fluorophores (e.g., 1-aminopyrene and 2-naphthaleneethanol), ionophores (e.g., 2-(aminomethyl)-15-crown-5), molecules susceptible to functional group conversion (e.g., benzyl amine), electroactive molecules (e.g., ferrocene methanol, 10H-(phenothiazine-10) propionic acid, anthraquinone-2-carboxylic acid), sugars (e.g.,  $\alpha$ -D-glucose), nucleotides (e.g., uridine), and mixtures thereof.

285 The coupling step is preferably carried out in the presence of a reagent which may be, for example, a phosphonium reagent, a facilitating reagent, as well as mixtures thereof. Preferably, the facilitating reagent may be selected from a base, a catalyst, and mixtures thereof. Preferred bases include various pyridine derivatives. Other examples of components which may be employed during the coupling step include, but are not limited to, BOP (benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate); HOBt (1-hydroxybenzotriazole); NMM (4-methylmorpholine); DMAP (4-dimethylaminopyridine); and mixtures thereof.

295 In another aspect, the invention relates to a method of analyzing a nanometer-sized particle. The method comprises subjecting a nanometer-sized particle as defined herein to an analytical technique such that the composition of the functional materials of the monolayer on the particle are determined. A number of analytical techniques may be employed in this method. Examples of 300 such techniques include, but are not limited to, NMR spectroscopy, electrochemical techniques, fluorescent emission spectroscopies, and infrared spectroscopies.

Examples

305 The examples which follow are set forth to illustrate the invention, and are not meant as a limitation thereon. Although the following examples explicitly employ only monolayer-protected nanometer-sized gold cores, other monolayer-protected nanometer-sized cores (comprising at least one metal or metal alloy which is selected from the group consisting of a semiconducting material, an 310 oxide material, a Group VIIIA element, a Group IB element, a Group IIB element, alloys thereof, and mixtures thereof) have reactivity and stability that allow their use with the reaction conditions set forth below; thus, one skilled in the art could reasonably expect that the examples set forth below would be applicable to all 320 embodiments set forth in the claims of this patent.

315 In the examples, 10H-(phenothiazine-10)propionic acid was synthesized according procedures set forth in Peek, B.M. et al., *Int. J. Peptide Protein Res.* 38 (1991) 114. 11-mercaptop-undecanoic acid and 11-mercaptop-undecanol were either synthesized according to a procedure described in Bain, C.D. et al., *J. Am. Chem. Soc.* 111 (1989) 321; or purchased from the Aldrich Chemical Company 320 of Milwaukee, Wisconsin (95% and 97% purity, respectively). Tetrahydrofuran (less than 16 ppm water content) was used for all coupling reactions. The synthesis of nanometer-sized gold cores which are stabilized by chemisorbed layers of dodecanethiolate was accomplished in accordance with a procedure described Hostetler, M.J., et al., *Langmuir* 14 (1998) 17-30 and addition of  $\omega$ -Z-325 alkanethiol (with Z being COOH or OH) onto the nanometer-sized gold cores was accomplished as described in Hostetler, et al., *J. Am. Chem. Soc.* 118 (1996) 4212-4213. All other reagents were used as received. Nomenclature for the nanometer-sized gold cores protected by a complex monolayer may be defined as CX:CYZ (a:b) wherein X specifies the length of the alkanethiol chain, 330 Y specifies the chain length of the place exchanged  $\omega$ -Z-alkanethiol (with Z being COOH or OH) and (a:b) specifies the mole ratio of X and Y chains in the monolayer on the nanometer-sized gold core as determined from the methyl/CH<sub>2</sub>R <sup>1</sup>H NMR spectral ratio in solutions of nanometer-sized gold cores

335 protected by complex monolayers that have been treated with I<sub>2</sub> in order to remove the monolayer from the nanometer-sized gold core as taught in Templeton, A.C., et al., *J. Am. Chem. Soc.*, **120** (1998) 1906-1911. Percent conversion data was determined using the aforementioned methodology.

340 With respect to the spectroscopy data, <sup>1</sup>H NMR spectra (in C<sub>6</sub>D<sub>6</sub>, CD<sub>2</sub>Cl<sub>2</sub>, or CDCl<sub>3</sub>) were obtained using a Bruker AMX 200 MHz spectrometer sold by Bruker Instruments, Inc. of Billerica, Massachusetts. A line broadening factor of 1Hz was used to improve the S/N of the NMR resonances belonging to the monolayer on the nanometer-sized gold core. Infrared absorbence spectra of clusters as thin films were acquired using a Biorad 6000 FTIR spectrometer sold by Bio-Rad Laboratories, Inc. of Cambridge, Massachusetts. Reported IR bands 345 are those that are unique to each functionalized monolayer-protected nanometer-sized gold core (an infrared spectra of a nanometer-sized gold core which is stabilized by chemisorbed layers of dodecanethiolate was used for background subtraction).

350 The examples set forth below employ the following abbreviations for the sake of brevity: THF (tetrahydrofuran); DMF (dimethylformamide); MPC (monolayer-protected nanometer-sized gold core); BOP (benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate); HOBt (1-hydroxybenzotriazole); NMM (4-methylmorpholine); DMAP (4-dimethylaminopyridine); 4-amino-TEMPO (4-amino-2,2,6,6-tetramethylpiperidinyloxy, free radical); IR (infrared); NMR (nuclear magnetic resonance); EPR (electron paramagnetic resonance); ml (milliliter); mg (milligram); ppm (parts per million); cm<sup>-1</sup> (wavenumbers); d<sup>+</sup> (symmetric methylene stretching vibration); d<sup>-</sup> (antisymmetric methylene stretching vibration); br (broad. All reactions were performed within the temperature range 15 to 35 °C, although one skilled in the art would reasonably assume that the reactions 360 would also be functional in the temperature range -80 to 100 °C.

Examples 1-6**Coupling of Amines to C12:C11COOH (a:b)**

365 Various coupling reactions were performed according to a procedure set forth in McCafferty et al., *Tetrahedron* **51** (1995) 1993. In these examples, ca. 100 mg of acid MPC (C12:C11COOH MPC (4:1)) was treated with five equivalents (relative to moles of MPC acid groups) of BOP (60 mg), HOBt (18 mg), NMM (14 uL), and DMAP (16 mg) in low water THF (concentration of 2 mg cluster/mL). Following a brief activation period (10 minutes), five equivalents of a specified amine was added to the reaction mixture and the solution was stirred at room temperature for 15 hours. The solvent was then removed under vacuum and the reacted MPC was collected on a frit where unreacted materials were removed by washing with 500 mL of acetonitrile followed by sonication/decanting 370 with 50 mL acetonitrile (3X).

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Example 1**4-amino-TEMPO (spin label)**

380 The above procedure was carried out using 4-amino-TEMPO as the amine. The following data were obtained: IR: 2851 (d<sup>+</sup>), 2922 (d<sup>-</sup>), 1641, 1537 cm<sup>-1</sup>. The EPR spectrum is shown in Figure 2. The percent conversion to number coupled ratio was determined to be 95/13.

Example 2**4-(aminomethyl)-pyridine (metal ligand)**

385 The above procedure was carried out using 4-(aminomethyl)-pyridine as the amine. The following data were obtained; NMR (in CD<sub>2</sub>Cl<sub>2</sub>): δ(ppm) = 0.89 (br, 15.6 H), 1.28 (br, H), 1.74 (br, H), 2.21 (br, 3.1 H), 4.32 (br, 1.9 H), 7.12 (br, 2.3 H), 8.43 (br, 2 H). IR: 2850 (d<sup>+</sup>), 2920 (d<sup>-</sup>), 1653, 1602, 1539 cm<sup>-1</sup>. The 390 percent conversion to number coupled ratio was determined to be 95/9.5.

Example 3**glutamic acid di-*t*-butyl ester (amino acid)**

The above procedure was carried out using glutamic acid di-*t*-butyl ester  
395 as the amine. The following data were obtained: NMR (in C<sub>6</sub>D<sub>6</sub>): δ (ppm) = 1.02  
(br, 27 H), 1.4 (br, 218 H), 2.3 (br, 28 H), 4.8 (br, 2 H). IR: 2850 (d<sup>+</sup>), 2920 (d<sup>-</sup>),  
1734, 1680, 1650, 1536, 1392, 1367, 1155 cm<sup>-1</sup>. The percent conversion to  
number coupled ratio was determined to be 90/4.

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Example 4**1-aminopyrene (chromophore; fluorophore)**

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The above procedure was carried out using 1-aminopyrene as the amine.  
The following data were obtained: NMR (in CD<sub>2</sub>Cl<sub>2</sub>): δ (ppm) = 0.85 (br, 3 H), 1.3  
(br, 15 H), 1.85 (br, 1.9 H), 3.35 (br, 0.33 H), 3.55 (br, 0.32 H), 7.15 (br, 0.02 H),  
7.45 (br, 0.04 H), 7.7 (br, 0.06 H), 8.0 (br, 0.18 H). IR: 2851 (d<sup>+</sup>), 2921 (d<sup>-</sup>), 1735,  
1700, 1655, 1601, 1558, 1517 cm<sup>-1</sup>. The percent conversion to number coupled  
ratio was determined to be 95/7.

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Example 5**2-(aminomethyl)-15-crown-5 (ionophore)**

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The above procedure was carried out using 2-(aminomethyl)-15-crown-5  
as the amine. The following data were obtained: NMR (in C<sub>6</sub>D<sub>6</sub>): δ (ppm) = 0.75  
(br, 0.8 H), 1.0 (br, 3 H), 1.45 (br, 18 H), 2.25 (br, 2 H), 3.6 (br, 3.8 H). IR: 2949  
(d<sup>+</sup>), 2922 (d<sup>-</sup>), 1734, 1650, 1543, 1122 cm<sup>-1</sup>. The percent conversion to number  
coupled ratio was determined to be 80/8.

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Example 6**benzyl amine (group conversion)**

The above procedure was carried out using benzyl amine as the amine.  
The following data were obtained: NMR (in CD<sub>2</sub>Cl<sub>2</sub>): δ (ppm) = 0.90 (br, 31 H),

1.3 (br, 141 H), 2.2 (br, 4 H), 4.4 (br, 3 H), 7.3 (br, 5 H). IR: 2850 (d<sup>+</sup>), 2920 (d<sup>-</sup>), 1734, 1646, 1547 cm<sup>-1</sup>). The percent conversion to number coupled ratio was determined to be 95/7.

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#### Examples 7-10

##### **Coupling of alcohols to C12:C11COOH (a:b)**

In reactions set forth in these examples, ca. 100 mg of the acid MPC (C12: C11COOH (4:1)) was treated with five equivalents (relative to the moles of MPC acid groups) of BOP (60 mg), HOBT (18 mg), NMM (14  $\mu$ and DMAP (16 mg) in low water THF (conc. of 2 mg cluster/mL). Following a brief activation period (10 minutes), five equivalents of an alcohol described in these examples was added to the reaction mixture and the solution was stirred at room temperature for 15 hours. The solvent was removed under vacuum and the reacted MPC was collected on a frit where unreacted materials were removed by washing with 500 mL of acetonitrile followed by sonication/decanting with 50 mL of acetonitrile (3X).

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#### Example 7

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##### **2-naphthalene-ethanol (chromophore)**

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The above procedure was carried out using 2-naphthalene-ethanol as the alcohol. The following data were obtained: NMR (in CD<sub>2</sub>Cl<sub>2</sub>):  $\delta$  (ppm) = 0.92 (br, 10 H), 1.3 (br, 69 H), 2.25 (br, 2 H), 3.4 (br, 1.1 H), 3.6 (br, 2 H), 5.9 (br, 0.10 H), 6.5 (br, 0.11 H), 7.15 (br, 0.05 H), 7.4 (br, 0.17 H), 7.8 (br, 0.18 H) IR: 2851 (d<sup>+</sup>), 2922 (d<sup>-</sup>), 1739, 1653, 1118, 1070 cm<sup>-1</sup>. The percent conversion to number coupled ratio was determined to be 85/11.5.

#### Example 8

##### **Ferrocene-methanol (electroactive)**

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The above procedure was carried out using ferrocene-methanol as the alcohol. The following data were obtained: NMR (in C<sub>6</sub>D<sub>6</sub>):  $\delta$  (ppm) = 1.0 (br, 27

H), 1.5 (br, 226 H), 3.9 (br, 5 H), 4.2 (br, 1.3 H), 4.8 (br, 0.9 H) IR: 2851 (d<sup>+</sup>), 2922 (d<sup>-</sup>), 1727, 1680, 1594, 1268, 1244 IR: 2851 (d<sup>+</sup>), 2922 (d<sup>-</sup>), 1737, 1710, 1653, 1616 cm<sup>-1</sup>. The percent conversion to number coupled ratio was

455 determined to be 50/5.

### Example 9

#### **α-D-glucose (sugar)**

The same procedure was employed except that α-D-glucose was used and a solvent 5:1 THF/DMF was employed to aid glucose solubility. The following data were obtained: NMR (in CD<sub>2</sub>Cl<sub>2</sub>): δ (ppm) = 0.9 (br, 3 H), 1.3 (br, 17 H), 2.9 (br, 0.34 H) IR: 2850 (d<sup>+</sup>), 2920 (d<sup>-</sup>), 1815, 1733, 1647, 1612, 840, 781, 769, 740 cm<sup>-1</sup>. The percent conversion to number coupled ratio was determined to be 35/5.

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### Example 10

#### **Uridine (nucleotide)**

The same procedure was employed except uridine was used and a solvent 5:1 THF/DMF was employed to aid uridine solubility. The following data were obtained: NMR (in CD<sub>2</sub>Cl<sub>2</sub>): δ (ppm) = 0.9 (br, 3 H), 1.3 (br, 17 H), 2.9 (br, 0.85 H) IR: 2851 (d<sup>+</sup>), 2921 (d<sup>-</sup>), 1817, 1733, 1700, 1633, 1616, 1491, 1411, 843, 782, 764, 742 cm<sup>-1</sup>. The percent conversion to number coupled ratio was determined to be 60/8.

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### Examples 11-12

#### **Coupling of carboxylic acids to C12:C11OH (a:b)**

In these examples, five equivalents (relative to moles of MPC alcohol) of a carboxylic acid was treated with five equivalents of BOP (60 mg), HOEt (18 mg), NMM (14 μL), and DMAP (16 mg) in low water THF (conc. of 2 mg cluster/mL).

480 Following a brief activation period (10 minutes), ca. 100 mg of the alcohol (C12:C11OH (4:1)) was added and the solution was stirred at room temperature

for 15 hours. The solvent was removed under vacuum and the reacted MPC was collected on a frit where unreacted materials were removed by washing with 500 mL of acetonitrile followed by sonication/decanting with 50 mL acetonitrile (3X).

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### Example 11

#### **10H-(phenothiazine-10)propionic acid (electroactive)**

The above procedure was carried out using 10H-(phenothiazine-10)propionic acid as the acid. The following data were obtained: NMR (in CD<sub>2</sub>Cl<sub>2</sub>): δ (ppm) – 0.9 (br, 17 H), 1.3 (br, 150 H), 2.75 (br, 0.2 H), 4.0 (br, 2 H), 4.1 (br, 2 H), 6.8 (br, 5.9 H), 7.1 (br, 5.9 H) IR: 2851 (d<sup>+</sup>), 2921 (d<sup>-</sup>), 1817, 1733, 1700, 1633, 1616, 1491, 1411, 843, 782, 764, 742 cm<sup>-1</sup>. The electrochemistry of this MPC derivative is shown in Figure 3a. The percent conversion to number coupled ratio was determined to be 65/7.4.

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### Example 12

#### **Anthraquinone-2-carboxylic acid (electroactive; chromophore)**

The above procedure was carried out using anthraquinone-2-carboxylic acid as the acid. The following data were obtained: NMR (in CD<sub>2</sub>Cl<sub>2</sub>): δ (ppm) = 0.88 (br, 17 H), 1.3 (br, 119 H), 1.8 (br, 14 H), 3.55 (br, 1.8 H), 4.3 (br, 2 H), 7.8 (br, 1.8), 8.3 br, 3.4 H), 8.8 (br, 0.93 H) IR: 2851 (d<sup>+</sup>), 2922 (d<sup>-</sup>), 1727, 1680, 1594, 1268, 1244 cm<sup>-1</sup>. The percent conversion to number coupled ratio was determined to be 75/7.5.

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### Example 13

#### **Coupling of BOC-phenylalanine**

Five molar equivalents of BOC-phenylalanine (168 mg) were activated (10 minutes) by treatment with five equivalents of BOP (280 mg), HOEt (87 mg), NMM (128 μL), and DMAP (77 mg) in THF (conc. of 2 mg cluster/mL). 200 mg

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of 3:1 C12:C111OH was added to the above and the solution was stirred at room temperature for 15 hours. The solvent was removed under vacuum and the reacted MPC was collected on a frit where unreacted materials were removed by washing with 500 mL of acetonitrile followed by sonication/decanting with 50 mL acetonitrile (3X). The following data were obtained: NMR (in C<sub>6</sub>D<sub>6</sub>): δ (ppm) = 0.88 (br, 12.8 H), 1.35 (br, 48 H), 3.05 (br, 1.75 H), 3.55 (br, 2.2 H), 4.1 (br, 1.7 H), 4.5 (br, 0.3 H), 7.2 (d, 5 H).

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#### Example 14

##### **Deprotection of BOC-Phe-terminated MPC.**

To a 30 ml solution of 1.0 g of BOC-Phe-terminated MPC (synthesized in Example 14) in CH<sub>2</sub>Cl<sub>2</sub> was added 7.5 ml of trifluoroacetic acid. The reaction 525 was stirred at room temperature for 2 hours after which time the reaction mixture was diluted with 100 ml of distilled H<sub>2</sub>O. The organic phase was separated, and the aqueous phase was further washed with 100 ml of CH<sub>2</sub>Cl<sub>2</sub>. The combined organic phases were washed with 2 x 100 ml of 10% NaHCO<sub>3</sub> and then removed in vacuo. The precipitate was then washed with copious quantities of acetonitrile 530 and was air dried. The following data were collected: NMR (in CD<sub>2</sub>Cl<sub>2</sub>): δ (ppm) = 0.88 (br, 26 H), 1.35 (br, 192 H), 7.2 (d, 5 H).

#### Example 15

##### **Coupling of BOC-alanine**

535 BOC-alanine was coupled to the C-terminus of phenylalanine as described in Examples 2-8 (five molar excess of reagents). The following data were obtained; NMR (in C<sub>6</sub>D<sub>6</sub>): δ (ppm) = 0.85 (br, 13.6 H), 1.35 (br, 115 H), 3.05 (br, 2.6 H), 4.1 (br, 2.5 H), 4.7 (br, 0.6 H), 7.2 (d, 5 H).

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Example 16**Deprotection of BOC-Ala-Phe-terminated MPC**

BOC-alanine was deprotected as described in Example 15. The following data were obtained: NMR (in CD<sub>2</sub>Cl<sub>2</sub>): δ (ppm) = 0.85 (br, 16.8 H), 1.35 (br, 156 H), 3.05 (br, 3.4 H), 4.1 (br, 1.9 H), 4.7 (br, 0.7 H), 7.2 (d, 5 H) 7.65 (br, 0.12 H).

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Example 17**Coupling of BOC-isoleucine**

BOC-isoleucine was coupled to the C-terminus of alanine as described in Examples 2-8 above (five molar excess of reagents). The following data were obtained: NMR (in C<sub>6</sub>D<sub>6</sub>): δ (ppm) = 0.85 (br, 18 H), 1.35 (br, 147 H), 3.05 (br, 2.6 H), 4.1 (br, 2.4 H), 4.7 (br, 0.02 H), 7.2 (d, 5 H). IR: 3063, 3029, 2848 (d<sup>+</sup>), 2917 (d<sup>-</sup>), 1738, 1718, 1687, 1644, 1513, 1497, 1164 cm<sup>-1</sup>.

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Example 18**Deprotection of BOC-Ile-Ala-Phe-terminated MPC**

BOC-isoleucine was deprotected as described in Example 15. The following data were obtained: NMR (in CD<sub>2</sub>Cl<sub>2</sub>): δ (ppm) = 0.85 (br, 16.8 H), 1.35 (br, 156 H), 3.05 (br, 3.4 H), 4.1 (br, 1.9 H), 4.7 (br, 0.7 H), 7.2 (d, 5 H) 7.65 (br, 0.12 H).

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Example 19**Analysis of  $\omega$ -Carboxylic Acid-Alkanethiolate Functionalized MPCs using I<sub>2</sub> Decomposition**

565 Approximately 50 mg of the  $\omega$ -carboxylic acid-alkanethiolate functionalized MPC was dissolved in dichloromethane and stirred with approximately 3 mg of iodine for one hour. Following disulfide formation, which was monitored by a change in solution color from dark brown to clear violet, the insoluble brown residue (actual identity of insoluble materials not identified) was removed and the sample rotovapped to dryness. The NMR of the I<sub>2</sub>-

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decomposed C12:C11COOH (4:1) (in  $\text{CDCl}_3$ ) was:  $\delta$  (ppm) = 0.85 (t, 5.5 H), 1.25 (m, 35.4 H), 1.66 (m, 5.3 H), 2.35 (t, 1 H), 2.65 (t, 4 H).

#### Example 20

##### **Electrochemical Measurement: Cyclic Voltammetry**

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Cyclic voltammetry (seen in Figure 2) was performed with a BAS 100B electrochemical analyzer sold by Bioanalytical Systems, Inc., located in West Lafayette, Indiana. A platinum 3 mm diameter working electrode was polished with 0.5  $\mu\text{m}$  diamond (Buehler) paste followed by rinsing with water, ethanol, and acetone prior to each experiment. A Pt coil counter electrode and saturated calomel reference electrode resided in the same cell compartment as the working electrode. Solutions (2:1 toluene/acetonitrile as disclosed in Ingram et al., *J.Am.Chem.Soc.* **1996**, 51, 1093) of the phenothiazine-MPC (0.8 mM in phenothiazine) and of phenothiazine monomer (1 mM) were degassed and then bathed throughout with solvent-saturated  $\text{N}_2$ .

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#### Example 21

##### **Electrochemical Measurement: Thin-layer coulometry**

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Thin-layer coulometry (as seen in Figure 2b) was performed using a BAS 100B electrochemical analyzer. A 4.3 mm diameter Pt working electrode was polished with 0.5  $\mu\text{m}$  of diamond (Buehler) paste followed by rinsing with water, ethanol, and acetone and toluene prior to each experiment. A Pt wire counter electrode and Ag wire quasi-reference electrode (AgQRE) resided in a locally designed thin-layer cell as defined in Reilley, C.N., *Pure Appl. Chem.* **18** (1968) 137. A Mitutoyo digital micrometer (1-2", 0.00005" resolution) sold by Mitutoyo Corporation of Japan was fitted to the cell and used to define the thin-layer cell thickness, L. Charge-time measurements were performed for cell thicknesses of 2-30  $\mu\text{m}$ , and Q values of zero-time intercepts extrapolated from the longer time plateaus (20-32 s) of each charge-time plot. The product of the number of phenothiazines per cluster, 0, and of electrons per phenothiazine, n, is obtained

from the slope of a Q versus L plot as shown in Figure 3b ( $Q/L = nFAC = 11.47 \times 10^{-3} \text{ C/cm}^2$ ,  $C = 1.07 \times 10^{-7} \text{ mole/cm}^3$ ).

In the drawings, specification, and examples there have been disclosed typical preferred embodiments of the invention and, although specific terms are employed, they are used in a generic and descriptive sense only and not for the purposes of limitation, the scope of the invention being set forth in the following claims.

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## THAT WHICH IS CLAIMED:

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1. A functionalized nanometer-sized particle comprising:  
a core comprising at least one metal or metal alloy; and  
a monolayer chemically bonded to said core, said monolayer containing at  
least one reactive substituent and wherein at least one reactive substituent is  
coupled to a functional material such that the monolayer is chemically modified.

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2. The particle according to Claim 1, further including amide or ester  
linkages which couple at least one reactive substituent and the functional  
material together.

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3. The particle according to Claim 1, wherein said at least one metal or metal  
alloy is selected from the group consisting of a semiconducting material, a metal  
oxide material, a Group VIIIA element, a Group IB element, a Group IIB element,  
alloys thereof, and mixtures thereof.

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4. The particle according to Claim 1, wherein said at least one metal or metal  
alloy is preferably selected from the group consisting of a Group VIIIA element, a  
Group IB element, a Group IIB element, alloys thereof, and mixtures thereof.

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5. The particle according to Claim 1, wherein said core has a diameter  
ranging from about 1 nm to about 999 nm.

6. The particle according to Claim 1, wherein said core has a diameter  
ranging from about 1 nm to about 100 nm.

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7. The particle according to Claim 1, wherein said monolayer is formed  
during the formation of said core.

640 8. The particle according to Claim 1, wherein said monolayer comprises a material selected from the group consisting of an organic compound, an inorganic compound, an organometallic compound, a biochemical compound, and mixtures thereof.

645 9. The particle according to Claim 1, wherein said monolayer comprises substituents selected from the group consisting of straight-chained molecules, branched molecules, hyperbranched molecules, compounds containing functional groups, and mixtures thereof.

650 10. The particle according to Claim 1, wherein said chemically modified monolayer includes materials selected from the group consisting of nonreactive materials, partially reactive materials, and mixtures thereof.

655 11. The particle according to Claim 1, wherein said chemically modified monolayer is chemically bonded to said core by a bond selected from the group consisting of a core-element-sulfur bond, a core-element-oxygen bond, a core-element-nitrogen-bond, core-element-phosphorus, core-element-boron, and combinations thereof.

660 12. The particle according to Claim 1, wherein said monolayer comprises at least one alkanethiol compound or derivative thereof.

13. The particle according to Claim 1, wherein at least one reactive substituent comprises at least one compound having the general formula:

$$R_n(EH)_x$$

665 wherein R is selected from the group consisting of an organic compound, an inorganic compound, an organometallic compound, a biochemical compound, and mixtures thereof; E is selected from the group consisting of S, O, NH<sub>2</sub>, NH,

CO<sub>2</sub>, SO<sub>3</sub>OH, PO<sub>2</sub>(OH)<sub>2</sub>, BO(OH)<sub>2</sub>, and mixtures thereof; n is an integer ranging from 1 to 5; and x is an integer ranging from 1 to 10.

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14. The particle according to Claim 1, wherein the at least one reactive substituent is selected from the group consisting of SH, OH, NH<sub>2</sub>, NH, CO<sub>2</sub>H, SO<sub>3</sub>OH, PO<sub>2</sub>(OH)<sub>2</sub>, BO(OH)<sub>2</sub>, and mixtures thereof.

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15. The particle according to Claim 1, wherein the at least one reactive substituent is selected from the group consisting of OH, NH<sub>2</sub>, NH, CO<sub>2</sub>H, and mixtures thereof.

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16. The particle according to Claim 1, wherein the at least one functional material coupled to said monolayer comprises functionality selected from the group consisting of SH, OH, NH<sub>2</sub>, NH, CO<sub>2</sub>H, SO<sub>3</sub>OH, PO<sub>2</sub>(OH)<sub>2</sub>, BO(OH)<sub>2</sub>, and mixtures thereof.

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17. The particle according to Claim 1, wherein the at least one functional material coupled to said monolayer comprises functionality selected from the group consisting of OH, NH<sub>2</sub>, NH, CO<sub>2</sub>H, and mixtures thereof.

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18. The particle according to Claim 1, wherein the functional material coupled to said monolayer is a catalyst.

19. The particle according to Claim 1, wherein the functional material coupled to said monolayer is a biomaterial.

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20. The particle according to Claim 1, wherein the functional material coupled to said monolayer has a low-lying excited state which is capable of undergoing fluorescence or electron-transfer when excited.

700 21. The particle according to Claim 1, wherein the functional material coupled to said monolayer is electrochemically active.

22. A solvent containing a nanometer-sized particle as recited in Claim 1 dissolved therein.

705 23. A method of making a functionalized nanometer-sized particle, said method comprising:

providing a nanometer-sized particle comprising a core which comprises at least one metal or metal alloy, and a monolayer adsorbed onto core wherein said monolayer includes at least one reactive substituent; and  
710 coupling the nanometer-sized particle with a functional material such the monolayer is chemically modified.

24. The method according to Claim 23, wherein at least one reactive substituent comprises at least one compound having the general formula:

715  $R_n(EH)_x$

wherein R is selected from the group consisting of an organic compound, an inorganic compound, an organometallic compound, a biochemical compound, and mixtures thereof; E is selected from the group consisting of S, O, NH<sub>2</sub>, NH, CO<sub>2</sub>, SO<sub>3</sub>OH, PO<sub>2</sub>(OH)<sub>2</sub>, BO(OH)<sub>2</sub>, and mixtures thereof; n is an integer ranging from 1 to 5; and x is an integer ranging from 1 to 10.

720 25. The method according to Claim 23, wherein the functional material comprises at least one compound having the general formula:

$R_n(EH)_x$

725 wherein R is selected from the group consisting of an organic compound, an inorganic compound, an organometallic compound, a biochemical compound, and mixtures thereof; E is selected from the group consisting of S, O, NH<sub>2</sub>, NH,

CO<sub>2</sub>, SO<sub>3</sub>OH, PO<sub>2</sub>(OH)<sub>2</sub>, BO(OH)<sub>2</sub>, and mixtures thereof; n is an integer ranging from 1 to 5; and x is an integer ranging from 1 to 10.

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26. The particle according to Claim 25, wherein E is selected from the group consisting of O, NH<sub>2</sub>, NH, CO<sub>2</sub>; n is an integer ranging from 1 to 2; and x is an integer ranging from 1 to 3.

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27. The method according to Claim 23, wherein the functional material is selected from the group consisting of spin labels, metal ligands, amino acids, chromophores, fluorophores, ionophores, molecules susceptible to functional group conversion, electroactive molecules, sugars, nucleotides, and mixtures thereof.

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28. The method according to Claim 23, wherein said coupling step is carried out in the presence of a reagent selected from the group consisting of a phosphonium reagent, a facilitating reagent, and mixtures thereof.

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29. The method according to Claim 28, wherein the facilitating reagent is selected from the group consisting of a base, a catalyst, and mixtures thereof.

30. The method according to Claim 29, wherein the base is a pyridine derivative.

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31. The method according to Claim 23, wherein said coupling step is carried out in the presence of a component selected from the group consisting of benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate; 1-hydroxybenzotriazole; 4-methylmorpholine; 4-dimethylaminopyridine; and mixtures thereof.

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32. A method of analyzing a nanometer-sized particle, said method comprising:  
subjecting a nanometer-sized particle as defined in Claim 1 to an analytical  
760 technique such that the composition of the functional materials of the monolayer  
on said particle is determined.

765 33. The method according to Claim 32, wherein the analytical technique is

selected from the group consisting of NMR spectroscopy, electrochemical  
techniques, fluorescent emission spectropies, and infrared spectroscopy.

FIG. 1

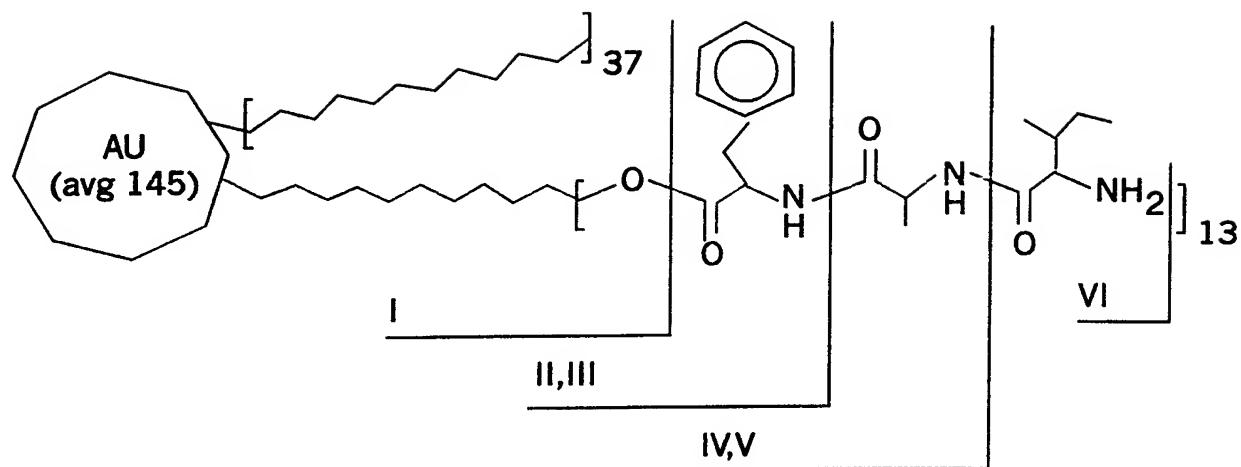


FIG. 2b

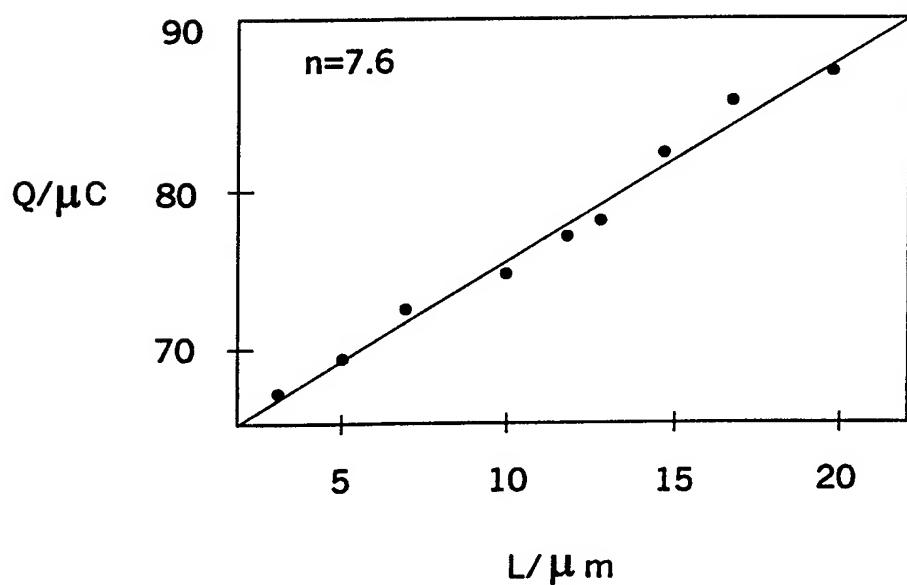


FIG. 2a

